Autologous Red Blood Cell Reinfusion: Effects on Stress and Fluid Regulatory Hormones During Exercise in the Heat

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This study assessed the effects of induced erythrocythemia on stress and fluid regulatory hormones during walking exercise in the heat. Six unacclimated male subjects received approximately 600 ml of a sterile saline solution containing 50% volume-to-volume of autologous erythrocytes. Three heat stress tests (HSTs) were attempted: one approximately 2 weeks prior to the reinfusion procedure, a second 48 h after the reinfusion procedure, and a third 1 week later, corresponding to 9 d subsequent to reinfusion. Each HST comprised three consecutive 45-min exercise and 15-min rest intervals ($\dot{V}O_2$ ~2.0 L·min^{-1}, 1.56 m·s^{-1}, 6% incline, 35°C, 45% rh). Blood was withdrawn before the HST and 30 min into each exercise (EX) bout. In all three HST's plasma cortisol (PC) levels were significantly ($p < 0.01$) reduced during the first EX bout compared to preexercise levels, and then progressively increased during the second and third EX intervals during HST 1. During HST 2 (48 h postinfusion), however, PC levels were significantly ($p < 0.05$) reduced in two blood samples (EX 2, 3) compared to the same blood samples from HST-1 (preinfusion). Plasma renin activity (PRA) and aldosterone (ALD) were significantly ($p < 0.01$) increased by the exercise/heat stress, but were unaffected by erythrocythemia either 48 h or 9 d subsequent to reinfusion. PRA and ALD were correlated ($r = 0.84$, $p < 0.001$) under all conditions. We concluded from this study that acutely induced erythrocythemia reduced the stress response to consecutive exercise/heat intervals as manifested in PC responses during HST 2. However, alterations in the fluid regulatory hormones ALD and PRA were unaffected by erythrocythemia, probably because total blood volume was not altered by reinfusion.

THE USE OF AUTOLOGOUS erythrocyte reinfusion to improve physical performance has been evaluated during several environmental and exercise paradigms. For example, Buick *et al.* (1) reinfused approximately 900 ml of autologous erythrocytes and demonstrated that both maximal aerobic power ($\dot{V}O_{2max}$) and endurance capacity were significantly increased 24 h after reinfusion in a normothermic and normoxic environment. Robertson and coworkers (18) confirmed these findings under normoxic conditions and extended the studies to demonstrate that exercise tolerance and $\dot{V}O_{2max}$ under normobaric, hypoxic conditions were both significantly improved by autologous transfusion. The same group (17) reported that similar improvements were noted for female test subjects during cycle ergometry under normoxic conditions. Further, they noted that the physiological advantages conferred by autologous reinfusion persisted for up to 2 weeks.

Gledhill (10) concluded in a recent review that increased O_2 delivery to working muscles should increase physical performance if the following criteria are met: incremented blood viscosity does not significantly reduce cardiac output, blood flow distribution is unaffected, and the oxidative capacity of the working muscles is not limiting. While potential increases in total blood and plasma volume may also be implicated in the increased physical capacity of induced erythrocythemia (13), Gledhill argued that such effects may be labile and are probably normalized after the first 24 h of reinfusion, although prior investigations have not addressed these effects in reinfused subjects.

Despite the apparent interest in the use of autologous reinfusion to improve physical performance, we are unaware of any studies which have assessed the effects of reinfusion on the endocrinological responses to exercise in the heat, especially during the first 48 h and up to 9 d following reinfusion. For several years, we have studied the responses of plasma hormones to exercise/heat stress after the body fluid status of a test subject has been experimen-

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tally manipulated. Thus, we reported (7) that, while hypohydration by 5% of body weight elicited significant elevations in plasma renin activity and aldosterone prior to and during exercise in the heat, these increments were attenuated following heat acclimation. These results were consonant with theory, since heat acclimation is accompanied by significant increases in intravascular fluid volume (20,21). Analogously, we demonstrated (8) that heat acclimation also moderated the stress hormone response to exercise in the heat during hypohydration. Most recently, we have observed (9) that increased severity of hypohydration is accompanied by increased circulating levels of aldosterone, plasma renin activity, and cortisol. Our previous results generally indicated that experimental manipulations which increased blood/plasma volume (i.e. heat acclimation) tended to reduce the response of selected stress and fluid regulatory hormones to exercise in the heat while procedures which reduced blood/plasma volume (i.e. dehydration) increased the response of these hormones to the heat stress test.

In considering the beneficial effects of acute erythrocythemia induced by autologous erythrocyte infusion, it is apparent that the physiological strain of exercise in the heat may be reduced by either improved O_2-CO_2 systemic transport or to heat dissipation. If such benefits accrued subsequent to erythrocythemia, then responses of stress hormones to exercise in the heat may be attenuated, particularly in unacclimated subjects. Alternatively, since the fluid regulatory hormone responses are also dependent on such variables as plasma osmolality, sodium levels, and oncotic pressure, as well as blood/plasma volume, it is difficult to predict the intensity and the direction of the responses that may occur. Thus, the current study was designed to determine the effects of acutely induced erythrocythemia on the response of a representative stress hormone (plasma cortisol, PC) and fluid regulatory hormones (aldosterone, ALD, and angiotensin I as determined by plasma renin activity, PRA) to a heat stress test. Unacclimated test subjects participated so that the subjective and physiological effects of heat acclimation did not mask the potential effects of the reinfusion.

MATERIALS AND METHODS

Subjects: Six adult male test subjects (Ss) participated, all members of the same military unit, and thus all exposed to similar regimens of diet, activity, and environment through the duration of the study. Anthropometric measures for the experimental group were (mean ± S.D.): age, 30 ± 7 years; weight, 79 ± 9 kg, height, 182.3 ± 4.2 cm, and percent body fat, 15 ± 5. All test subjects received a written and oral description of the procedures and risks of the study, and signed a voluntary consent form signifying their agreement to participate. All volunteers reserved the right to withdraw from the study at any time without prejudice or retribution, but none elected to do so.

Phlebotomy and reinfusion: During the late fall and early winter, two units of blood (900 ml) were collected from each volunteer; the collection of each unit was separated by at least 6 weeks. Phlebotomy, blood processing, storage and, ultimately, reinfusion were done by personnel on the staff of the Naval Blood Research Laboratory, Boston, MA. Blood was collected in citrate-phosphate-dextrose, and was stored at 4°C for 2–5 d. The erythrocytes were then separated by centrifugation and suspended in 40% weight-to-volume of glycerol, deep-frozen to −80°C (22,23) and stored. At reinfusion, the glycerolized erythrocytes were thoroughly washed (Haemonetics Blood Processor 115) and resuspended in a saline-glucose-phosphate solution; approximately 600 ml of solution with autologous erythrocytes (50% HCT) were reinfused over a 1-h time period.

Heat stress tests: Three heat stress tests (HSTs) were conducted at an environmental temperature of 35°C and a relative humidity of 45%. Each HST comprised a total of 180 min (three repetitions of 45 min exercise, EX1, EX2, EX3, interspersed by 15 min rests) unless predetermined safety criteria (heart rate >180 bpm, rectal temperature >39.5°C) or exhaustion were achieved. The exercise component of the HST involved walking (1.56 $m \cdot s^{-1}$) on an inclined (6%) treadmill. During the rest intervals, Ss were reweighed and rehydrated with sufficient cool water to maintain initial body weight. A program of scheduled rehydration was also employed during each exercise bout. The subjects wore only shorts, socks, and tennis shoes during each HST. Each HST was conducted on three separate occasions: the first or control HST was completed at least 6 weeks after the second phlebotomy, and approximately 2 weeks prior to the autologous reinfusion during the late spring season; the second was accomplished exactly 48 h after completion of the reinfusion procedure; and the third HST was 1 week following the second, which corresponded to 9 d after the reinfusion procedure. Thus, the minimal time interval between HSTs was 1 week.

Blood sampling: Indwelling Teflon catheters were inserted in a superficial arm vein prior to each HST; their patency was maintained by flushing with heparinized saline. Preexercise blood samples of 10 ml were obtained after the Ss stood quietly in a moderate environment (antechamber, 20°C, 40% rh) for at least 20 min to control for postural effects on vascular fluid shifts (12). The remaining three 10-ml blood samples were obtained 30 min into each exercise bout (EX1, EX2, EX3) of the respective HST while the subjects continued to walk. Blood was centrifuged at 1000 G for 30 min, aliquotted, and frozen at −20°C for subsequent analysis. Tests were conducted between 0700 and 1100 hours to offset the effects of circadian variations on the dependent variables (14).

Plasma analyses: Samples were analyzed for PC using commercially prepared test kits purchased from New England Nuclear Corp., Billerica, MA, according to standardized procedures outlined in their technical bulletin. Using these techniques intra-assay variability determined in our laboratory was just 2.5% and inter-assay variability was 7.3%. PC values are generally reported to range from 4–25 $\mu g \cdot 100\ ml^{-1}$ depending importantly on the time of day at which the blood samples are drawn (14). Angiotensin I levels were assessed by quantifying PRA using radioimmunoassay test kits also produced by New England Nuclear Corp. When converting enzyme and angiotensinases are appropriately inhibited, it has been demonstrated that the accumulation of angiotensin I reflects plasma renin activity. Intra-assay variability was determined to be 4% and inter-assay variability was 7.2% by these methods. Control levels of PRA for healthy normotensive men range from approximately 1.0–4.0 ng Angiotensin I formed per hour per ml plasma by this method. Aldosterone (ALD) levels were quantified using radioimmunoassay test kits purchased

from Diagnostics Products Corp, Los Angeles, CA, by methods outlined in their technical bulletin. Intra-assay variability was 9.3% by these methods and interassay variability was recorded at 11%. Expected values for normotensive adult men range from 5–31 $ng \cdot dl^{-1}$ by these methods.

Statistics: Repeated measures analyses of variance were performed followed by the application of Tukey's *t* test corrected for multiple group comparisons to determine the effects of exercise/heat stress on the variables of interest (15,16). During the preinfusion HST, one test subject did not complete the third exercise bout; therefore, a single calculated value was used for each variable (15, p. 228). To determine the significance of effects of red cell reinfusion, Dunnett's *t* test (15, p. 422) for paired, dependent data was performed and the results for the preinfusion trial (HST1) were compared with those of the 48-h postinfusion HST (HST2) as well as the 9-d HST (HST3). Correlation coefficients were calculated by linear regression analysis. For all statistical tests, the null hypothesis was rejected at $p \leq 0.05$.

RESULTS

Fig. 1 illustrates the effects of erythrocyte reinfusion and exercise/heat stress on circulating levels of cortisol in these unacclimated test subjects. The results indicate that, during all three HSTs there occurred a reduction in PC levels between the preexercise and EX1 sample which was significant for the first (preinfusion) HST ($p = 0.05$) and the second (48-h postinfusion) HST ($p < 0.01$). However, during the third (9-d postinfusion) HST, PC was at an apparently low basal level in the preexercise sample (8.1 $\mu g \cdot dl^{-1}$) and was further reduced to 6.5 $\mu g \cdot dl^{-1}$ during EX1; the minimum difference necessary for significance was 4.36 $\mu g \cdot dl^{-1}$, and this change was not significant in the third HST. We have observed this decrease previously (8,9) and attributed the decrement to the normally occurring circadian reduction during this time of day. The cumulative effects of exercise in the heat apparently offset the anticipated continued circadian decline of PC until, by the third exercise interval in the preinfusion (HST1) and 9-d postinfusion (HST3) HSTs, PC levels were not significantly different from preexercise levels. However, in the 48-h postinfusion HST (HST2), PC levels were persistently and significantly ($p < 0.01$) depressed during all three exercise bouts from preexercise. The effects of induced erythrocythemia are best demonstrated if paired data are compared between the preinfusion (HST1) and the 48-h postinfusion (HST2) HSTs. In these comparisons, PC was significantly reduced at each of the exercise intervals in the 48-h postinfusion trial (HST2) vs. the respective sample of the preinfusion (HST1) trial ($p < 0.005$ EX2 and $p < 0.05$ EX1, EX3).

Values for aldosterone responses to reinfusion and exercise in the heat are noted in Fig. 2. The effects of exercise in the heat were apparent since, by the second exercise bout during all three HSTs, ALD levels were significantly ($p < 0.01$) elevated over preexercise concentrations. During EX1, ALD levels (vs. preexercise) were significantly elevated during the preinfusion ($p < 0.05$) and 48-h postinfusion ($p < 0.01$) HSTs; however, despite a consistent trend, significance was not achieved during EX1 for the 9-d postinfusion trial (preexercise level = 24.28 $ng \cdot dl^{-1}$, EX1 level = 34.4 $ng \cdot dl^{-1}$, minimum difference for significance = 13.8 $ng \cdot dl^{-1}$). Erythrocythemia, however, apparently had no effect on the ALD responses to exercise in the heat as no significant differences were noted in comparisons between pre- and 48-h or 9d-postinfusion HSTs.

Responses of plasma levels of PRA (Fig. 3) were remarkably consistent during all HSTs, with significant ($p < 0.01$) increments noted even during the first exercise period (vs. preexercise). Further, these elevations persisted; for all three HSTs, the levels measured during EX2 were significantly ($p < 0.01$) greater than those recorded during EX1 (HST1, 7.91 vs. 9.76; HST2, 6.71 vs. 9.20; HST3, 6.59 vs. 9.02 $ng \cdot ml^{-1} \cdot h^{-1}$). Examination of the data indicates that, during the third exercise interval, the rate of increase had moderated for all three HSTs. Fig. 2 and 3 illustrate apparently analogous responses of PRA and ALD to exercise in the

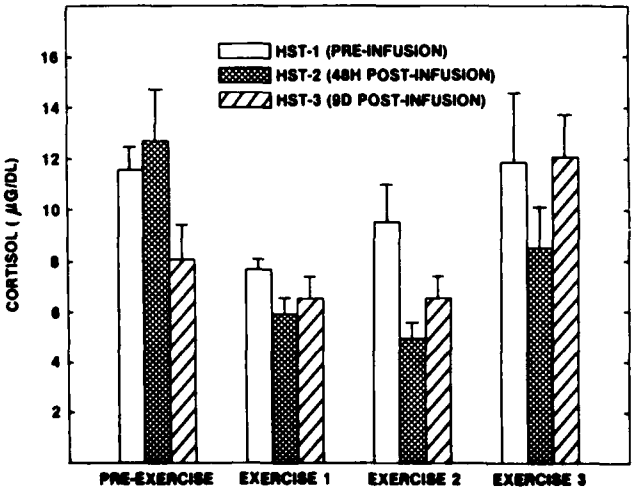

Fig. 1. Effects of acutely induced erythrocythemia and exercise in the heat on circulating cortisol levels. Means ± S.E.M. are depicted for n = 6 in all cases. Blood was removed after standing for 20 min in a moderate environment (20°C, 45% rh) for the pre-exercise sample, and 30 min into each exercise interval during the heat stress tests (1.56 $m \cdot s^{-1}$, 6% incline, 35°C, 45% rh).

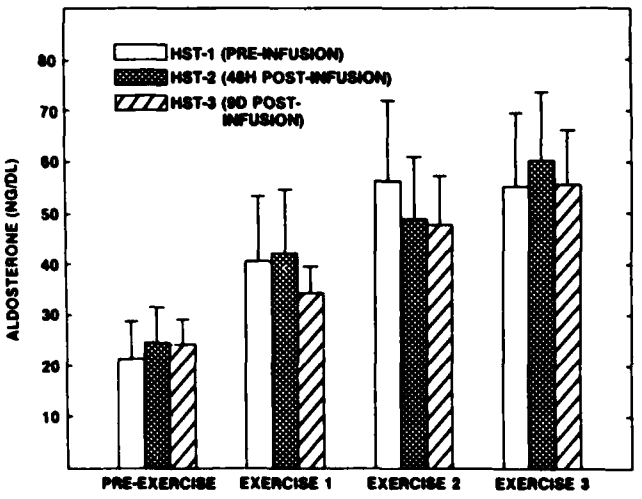

Fig. 2 Effects of erythrocythemia and exercise in the heat on circulating levels of aldosterone during exercise in a hot environment. All conditions and specifications as in Fig. 1.

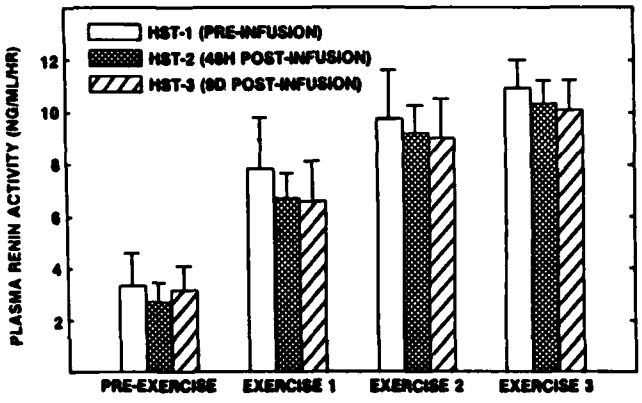

Fig. 3. Effects of erythrocythemia and exercise in the heat on circulating levels of plasma renin activity during exercise in the heat. All conditions and specifications as in Fig. 1.

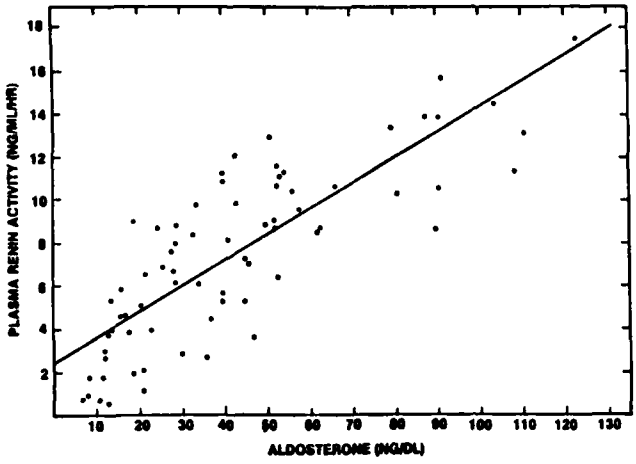

Fig. 4. Linear regression and scatter plot of values for PRA and ALD during all three heat stress tests and before and during all exercise bouts.

heat and erythrocythemia: statistical analysis confirmed the correlation between these two covariables: $n = 72$, $r = 0.84$, $t = 12.76$, $p < 0.001$ (Fig. 4).

DISCUSSION

We have previously reported (8,9) that when euhydrated subjects exercise in the heat under conditions similar to those selected in the current experiments, physiological stress response, as manifested in circulating cortisol levels, is minimal. In euhydrated subjects tested between 0700–1100 hours, the decrement between the preexercise and the EX1 and EX2 samples had been a consistent observation in our earlier studies (8,9). In the current investigation, wherein the metabolic rate had been increased from approximately 30% (8,9) to about 50% $\dot{V}O_{2max}$, PC levels increased in the preinfusion and 9-d postinfusion trials by the third exercise interval. These observations imply that, by the third exercise interval, Ss were experiencing physical discomfort as a result of the combination of the physical work rate and environment. Follenius *et al.* (6) had earlier reported that heat stress induced an adrenocortical response only in those individuals who experienced physical discomfort during the exposure.

Alternatively, erythrocyte reinfusion had repressive effects on this cumulative stress response; even during the first exercise interval, plasma levels of cortisol during the second HST (48-h postreinfusion) were significantly reduced by approximately 24% compared with the preinfusion trial. Further, these reductions persisted throughout the second and third exercise bouts. This may be interpreted in terms of an attenuation of the physiological strain of this exercise/heat regimen following reinfusion. It had been previously demonstrated that the increased arterial oxygen content induced by erythrocythemia can translate to a reduced requirement for skeletal muscle blood flow (24,25), in turn permitting greater cutaneous perfusion for heat dissipation. We have reported (19) that erythrocythemia not only provided a thermoregulatory benefit during exercise in the heat but also elicited an 11% increase in maximal O_2 consumption. Thus, it can be hypothesized that the reduced cortisol concentrations observed in the 48-h reinfusion trial could be a reflection of the reduced physiological strain engendered by this regimen of exercise in the heat which, during HST2, represents a reduced percentage of each subject's maximal aerobic capacity.

Levels of ALD and PRA were unaffected by the induced erythrocythemia during exercise/heat stress. We had originally hypothesized that an increased blood volume, anticipated particularly during the 48-h postinfusion HST (20), might alleviate the increments in ALD and PRA reported during and immediately subsequent to exercise in the heat (2,4,11). However, our hemodynamic measurements demonstrated (19) that, immediately prior to the second HST (48 h postinfusion), the acute erythrocythemia had elicited a marked (~7%) decrease in plasma volume compared to the preinfusion level, an observation which had not been reported previously. However, the increased erythrocyte volume was apparently offset by a compensatory decrement in plasma volume so that total blood volume was unchanged. Thus, the responses of the fluid regulatory hormones to exercise in the heat following erythrocythemia (Fig. 2, 3) are not inconsistent with our previous observations (7–9). The present results, with particularly sharp increases in PRA and ALD at each exercise interval in all three HSTs, confirm the intensity of these responses in unacclimated men. Our previous data (7,9), as well as that of other investigators (3–5), have indicated that the acquisition of heat acclimation moderates the intensity of the hormonal response to exercise in the heat. Clearly, induced erythrocythemia had no effects on the heat/exercise responses of these fluid regulatory hormones in our euhydrated, but unacclimated, subjects.

We have concluded from this study that autologous reinfusion of 2 units of erythrocytes attenuated the stress response to exercise in the heat as manifested in PC levels. Further, induced erythrocythemia had no effects on the incremental response pattern of the fluid regulatory hormones ALD and PRA to the heat/exercise regimen because the anticipated increase in blood volume effected by the red blood cell infusion was compensated by a slight decrease in plasma volume. Thus, the response pattern of the reinfused, euhydrated, and nonacclimated subjects is consistent with our earlier observations of response profiles in nonreinfused, euhydrated, and unacclimated test volunteers.

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